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*CURVATURE THEOREMS ON POLAR CURVES**

BY EDWARD KASNER AND JOHN DE CICCO
DEPARTMENT OF MATHEMATICS, COLUMBIA UNIVERSITY, NEW YORK

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1. The principle of duality in projective geometry is based on the theory of poles and polars with respect to a conic. For a conic, a point has only one kind of polar, the first polar, or polar straight line. However, for a general algebraic curve of higher degree n, a point has not only the first polar of degree $n - 1$, but also the second polar of degree $n - 2$, ..., the rth polar of degree $n - r$, ..., the $(n - 1)$ polar of degree 1. This last polar is a straight line. The general polar theory[1] goes back to Newton, and was developed by Bobillier, Cayley, Salmon, Clebsch, Aronhold, Clifford and Mayer.

For a given curve C_n of degree n, the first polar of any point 0 of the plane is a curve C_{n-1} of degree $n - 1$. If 0 is a point of C_n, it is well known that the polar curve C_{n-1} passes through 0 and touches the given curve C_n at 0. However, *the two curves do not have the same curvature.* We find that the ratio ρ_1 of the curvature of C_{n-1} to that of C_n is $\rho_1 = (n - 2)/(n - 1)$. For example, if the given curve is a cubic curve C_3, the first polar is a conic C_2, and at an ordinary point 0 of C_3, the ratio of the curvatures is 1/2. These simple theorems appear to be new.

We generalize this for all the higher polars. All the higher polars go through the given point 0 of the curve C_n and touch C_n at 0. In particular, the last polar coincides with the tangent line at 0. The curvatures at 0 all have different values (the last one being obviously zero), and we determine all these curvatures

In the above discussion, we assumed that 0 is an ordinary point of the algebraic curve C_n. We conclude by discussing also the theory at a singular point 0 of C_n; for example, inflections and cusps.

Although our new theorems are stated in metric terms, they are essentially theorems of differential projective geometry. This is a consequence of the theorem of Mehmke-Segre which states that if any two differ-

entiable curves are tangent at a given point 0, then the ratio ρ of their departures from the common tangent line is a projective invariant.

2. An algebraic curve C_n of degree n is defined in cartesian coördinates (x, y) by the equation

$$C_n: \ \varphi(x, y) = \sum_{k=0}^{n} P_k(x, y) = 0, \tag{1}$$

where $P_k(x, y)$ is a homogeneous polynomial in (x, y) of degree k, and $P_n(x, y)$ is not identically zero. It is convenient to use non-homogeneous cartesian coordinates (x, y) since we are dealing with the metric character of the polar curves.

Upon taking the given point 0 at the origin, we find that the first polar C_{n-1} of degree $n - 1$ of 0 with respect to C_n, is[2]

$$C_{n-1}: \ \sum_{k=0}^{n-1} (n - k)P_k(x, y) = 0. \tag{2}$$

The rth polar C_{n-r} of degree $n - r$ of the point 0 with respect to C_n may be defined by induction. It is the first polar of the same point 0 with respect to the $(r - 1)$ polar C_{n-r+1} of degree $n - r + 1$. Thus the equation of the rth polar of the origin 0 is

$$C_{n-r}: \ \sum_{k=0}^{n-r} (n - k)(n - k - 1)\ldots(n - k - r + 1)\, P_k(x, y) = 0. \tag{3}$$

The $(n - 1)$ polar C_1 of the origin 0 is the polar line

$$C_1: \ nP_0 + P_1(x, y) = 0. \tag{4}$$

3. Let the origin 0 be an ordinary point of the algebraic curve C_n so that $P_0 = 0$ and $P_1(x, y)$ is not identically zero. The tangent line of C_n at 0 is given by the equation $P_1(x, y) = 0$, and hence is identical with the polar line (4). By equations (2) and (3), it is seen that all the polar curves of 0 with respect to C_n pass through 0 and touch C_n at 0.

THEOREM 1. *The ratio ρ_1 of the curvature of the first polar C_{n-1} to the curvature of C_n, constructed at an ordinary point 0 of C_n, is*

$$\rho_1 = \frac{n - 2}{n - 1}. \tag{5}$$

This can be deduced from equations (1) and (2). By considering the equations (1) and (3), we obtain the following generalization. This can be also obtained by iteration.

THEOREM 2. *The ratio ρ_r of the curvature of the rth polar curve C_{n-r} to the curvature of the algebraic curve C_n, evaluated at an ordinary point 0 of C_n, is*

$$\rho_r = \frac{n - r - 1}{n - 1}. \tag{6}$$

In particular, consider a quartic curve C_4. At an ordinary point 0 of C_4, construct the polar cubic C_3, the polar conic C_2, and the polar line C_1. These all touch C_4 at 0 and the corresponding ratios of the curvatures are 2/3, 1/3, 0.

4. Before continuing with our work, it is necessary to consider some definitions. At a point P of a curve C, construct the tangent line t. From a point Q on the curve C near P, draw a line perpendicular to t and intersecting t in the point R. The *order of contact* γ of the curve C with its tangent line t at P is the positive number γ for which $\lim_{R \to P} \overline{RQ}/(\overline{PR})^{\gamma+1}$, is a finite non-zero number. It is remarked that γ is any positive real number.

Let two curves C_1 and C_2 be tangent at a point P. Through a point R on the common tangent line t near P, erect a perpendicular to t, which intersects the two curves C_1 and C_2 in the points Q_1 and Q_2, both of which are near P. The *ratio ρ of the departure of C_2 to that of C_1* from the common tangent line t is defined by the expression: $\rho = \lim_{R \to P} \overline{RQ_2}/\overline{RQ_1}$. If $\kappa^{(r)}$ denotes the rth derivative of the curvature κ of a curve C with respect to its arc length, and if γ, the order of contact of C_1 or C_2 with the common tangent line t at P, is a positive integer, then it can be shown that $\rho = \kappa_2^{(\gamma-1)}/\kappa_1^{(\gamma-1)}$. According to the theorem of Mehmke-Segre, *this ratio ρ is a projective invariant.*

5. By the equations (1), (2) and (3), it is found that if 0 is an ordinary point of the algebraic curve C_n, and if the order of contact of C_n with its tangent line at 0 is γ, then all the polar curves of 0 with respect to C_n are tangent to C_n at 0, and the order of contact of each of these polars with the common tangent line is also γ.

THEOREM 3. *The ratio ρ_r of the departure of the rth polar curve C_{n-r} to the departure of C_n, constructed at a point 0 of C_n in the case where the order of contact with the common tangent line at 0 is γ which may be any positive integer, is*

$$\rho_r = \frac{(n - \gamma - 1)(n - \gamma - 2)\ldots(n - \gamma - r)}{(n - 1)(n - 2)\ldots(n - r)}. \tag{7}$$

In particular, the corresponding ratios ρ_1, ρ_2, ρ_3 for the first, second and third polars are

$$\rho_1 = \frac{n - \gamma - 1}{n - 1}, \qquad \rho_2 = \frac{(n - \gamma - 1)(n - \gamma - 2)}{(n - 1)(n - 2)},$$

$$\rho_3 = \frac{(n - \gamma - 1)(n - \gamma - 2)(n - \gamma - 3)}{(n - 1)(n - 2)(n - 3)}. \tag{8}$$

6. Now we consider the case where the origin 0 is a cusp of lowest order of the algebraic curve C_n. Then in equation (1), we must have that $P_0 = 0$, $P_1(x, y)$ is identically zero, and $P_2(x, y)$ is a constant non-zero multiple of the square of a linear homogeneous form in (x, y). From equations (2) and (3), it is deduced that all the polars ($r = 1, 2, \ldots, n - 2$) have a cusp of the same qualitative nature at 0.

THEOREM 4. *The ratio ρ_r of the departure of the r th polar C_{n-r} to the departure of C_n, constructed at a simple cusp 0 of C_n in the case where the order of contact is 1/2, is*

$$\rho_r = \left(\frac{n - r - 2}{n - 2}\right)^{1/2}. \tag{9}$$

In particular, the corresponding ratios ρ_1, ρ_2, ρ_3 for the first, second and third polars are

$$\rho_1 = \left(\frac{n - 3}{n - 2}\right)^{1/2}, \quad \rho_2 = \left(\frac{n - 4}{n - 2}\right)^{1/2}, \quad \rho_3 = \left(\frac{n - 5}{n - 2}\right)^{1/2}. \tag{10}$$

7. In this section, we study the ratio ρ_r constructed at a simple cusp 0 of the algebraic curve C_n in the case where the order of contact with the common tangent line is greater than 1/2. We have proved the following result.

THEOREM 5. *Let 0 be a simple cusp of the algebraic curve C_n so that the order of contact γ of C_n with the tangent line at 0 is an integral multiple of 1/2. The r th polar C_{n-r} of 0 with respect to C_n also has a simple cusp at 0 with the same tangent line and the same order of contact γ at 0. If $\gamma = (2q - 1)/2$, the ratio ρ_r of the departure of C_{n-r} to the departure of C_n from the common tangent line at 0 is*

$$\rho_r = \left[\frac{(n - 2q - 1)(n - 2q - 2)\ldots(n - 2q - r)}{(n - 2)(n - 3)\ldots(n - 1 - r)}\right]^{1/2}. \tag{11}$$

In particular, the corresponding ratios ρ_1, ρ_2, ρ_3 for the first, second and third polars are

$$\rho_1 = \left(\frac{n - 2q - 1}{n - 2}\right)^{1/2}, \quad \rho_2 = \left[\frac{(n - 2q - 1)(n - 2q - 2)}{(n - 2)(n - 3)}\right]^{1/2},$$

$$\rho_3 = \left[\frac{(n - 2q - 1)(n - 2q - 2)(n - 2q - 3)}{(n - 2)(n - 3)(n - 4)}\right]^{1/2} \tag{12}$$

On the other hand, if γ is an integer, then ρ_r depends on the coefficients of the polynomial equation defining the algebraic curve C_n.

It is remarked that if 0 is a simple cusp where the order of contact is a positive integer γ, we meet for the first time a case where the ratio ρ_r is

not an arithmetic invariant but actually is a differential invariant, that is, ρ_r depends on the coefficients of the original algebraic curve C_n.

8. In our later work, we shall study this ratio ρ_r for a singular point 0 of higher order of the algebraic curve C_n. We shall generalize the results of this article to algebraic surfaces and to higher manifolds in later papers.

Our theorems since they deal with curvature appear to belong to differential geometry, that is, geometry in the small. But actually the notion of polar curve involves the total algebraic curve and therefore our theorems belong to geometry in the large, that is, to global geometry.

* Presented to the American Mathematical Society, Feb. 1947.

[1] Salmon, *Treatise on Higher Plane Curves* (1852).

[2] Kasner, "Determination of the Algebraic Curves Whose Polar Curves Are Parabolas," *Amer. Jour. Math.*, **26**, 164–168 (1903).

WEAK COMPACTNESS IN BANACH SPACES I

BY W. F. EBERLEIN

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF MICHIGAN

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1. In recent years the "weak" topology of Banach spaces has aroused interest, principally because a notion of weak compactness is vital to the theory of infinite dimensional linear spaces. Advances in the compactness theory, however, have been retarded by apparent dichotomy in nomenclature—"compactness" versus "bicompactness." From the standpoint of existing applications the basic tool is the weakly convergent subsequence or "compactness"; from the standpoint of topological structure the concept of "bicompactness" is fundamental. We prove that for weakly closed subsets of *any* Banach space these two seemingly disparate notions are one.

2. Let E be any Banach space with elements x and unit sphere S, E^* the conjugate space with elements f and unit sphere S^*, and E^{**} the second conjugate space with elements X. Denote by E_T the linear topological space (weak topology of E) formed by the elements of E under the neighborhood system $U(x_0) \equiv [x \mid |f_i(x - x_0)| < \epsilon, i = 1, \ldots, n]$, where the f_i are arbitrary elements of E^*. A like topology can be introduced in E^* by taking elements from E^{**}, but a more functional topological space ${}_TE^*$ (weak* topology of E^*) arises from the generic neighborhoods $U(f_0) \equiv [f \mid |f(x_i) - f_0(x_i)| < \epsilon, i = 1, \ldots, n]$, where the x_i are arbitrary elements of E. A space ${}_TE^{**} = {}_T(E^*)^*$ (weak* topology of E^{**}) is similarly generated; and the mapping $J(x) = X$ defined by $X(f) = f(x)$ imbeds E

isometrically in E^{**} and E_T in ${}_TE^{**}$. We shall regard this imbedding as implicitly performed whenever convenient. Our key lemma then takes the form of a necessary and sufficient condition that an element X of E^{**} lie in E.

The principal justification for the weak* topology is contained in the Tychonoff-Alaoglu[1] theorem: *S^* is a bicompact subset of ${}_TE^*$.* A further result we need is the equivalence[2] (for linear sets in E^*) of the properties of regular closure,[3] weak* closure, and, most important of all, "bounded" weak* closure—variously referred to as transfinite closure or weak* completeness. The contact with our work is the observation, implicit in the Banach[4] development, that an X of E^{**} lies in E if and only if the set $Q_X \equiv [f \mid X(f) = 0]$ is regularly closed in E^*.

We call a set M in E_T compact (weakly compact in E) if every infinite subset of M possesses at least one limit point x in E_T[5]; we call M sequentially compact if every infinite sequence in M contains a subsequence converging to a limit in E_T. Compactness implies the formally stronger property of sequential compactness[6] and the boundedness of M in the norm.[7]

LEMMA: *A necessary and sufficient condition that an element X of E^{**} lie in E is that there exist some weakly compact set M in E with the following property: Given arbitrary $(f_1, \ldots, f_n)$ in E^*, there is an x in M such that $X(f_i) = f_i(x)$ $(i = 1, \ldots, n)$.*

Proof: The necessity of the condition is trivial and, by virtue of the preceding remarks, establishing the sufficiency reduces to showing that if g in E^* is a weak* limit point of $Q_X \cdot S^*$, then $X(g) = 0$.

Let $\epsilon > 0$ be arbitrary. The hypothesis implies the existence of an x_0 in M such that $g(x_0) = X(g)$. But then there exists an f_1 in $Q_X \cdot S^*$ such that $|X(g) - f_1(x_0)| = |g(x_0) - f_1(x_0)| < \epsilon/2$. By alternate appeal to the condition on M and the definition of weak* limit point we thus construct a sequence (x_n) in M and a dual sequence (f_m) in $Q_X \cdot S^*$ with the properties:

(A) $||f_m|| \leq 1$ $\qquad (m = 1, 2, \ldots)$
(B) $f_m(x_n) = 0$ $\qquad (m = 1, \ldots, n)$
(C) $g(x_n) = X(g)$ $\qquad (n = 0, 1, \ldots)$
(D) $|X(g) - f_m(x_n)| < \epsilon/2$ $\qquad (n = 0, 1, \ldots, m - 1)$

The (sequential) weak compactness of M implies the existence of an x in E and a subsequence (x_{n_i}) of (x_n) such that $f(x) = \lim f(x_{n_i})$ for every f in E^*, whence $f_m(x) = 0$ for all m. A corollary to the fundamental theorem of Mazur[8]—on the equivalence of weak and strong closure for convex sets in E—provides an element z in E of the form $z = \sum_{n=0}^{N} a_n x_n$ $(a_n \geq 0, \sum_{n=0}^{N} a_n = 1)$ such that $||z - x|| < \epsilon/2$. Now set $m = N + 1$ in

(D). Multiplication of the $N + 1$ inequalities by the proper a_n, addition, and the triangle inequality yield $|X(g) - f_{N+1}(z)| < \epsilon/2$. But then

$$|X(g)| = |X(g) - f_{N+1}(z) + f_{N+1}(z) - f_{N+1}(x)| < \epsilon.$$

Hence $X(g) = 0$, and the proof of the lemma is complete.

If we consider a compact (and therefore strongly bounded) set M closed in E_T, we find that the lemma implies its closure[10] in ${}_TE^{**}$. The Tychonoff-Alaoglu result then yields the non-trivial half of the

THEOREM: *A set M in E_T is bicompact if and only if it is compact and closed.*

An automatic consequence of the lemma, by way of the Helly theorem,[9] is the

COROLLARY: *E is reflexive—$J(E) = E^{**}$—if and only if S is weakly compact.*

3. Our main result indicates that a number of special devices involving weak compactness can be replaced by a single bicompactness argument. For example, the mean ergodic theorem[11] for Abelian semi-groups of transformations reduces immediately to the r-parameter case, of which an integration-free treatment is possible. But deeper consequences in the ergodic theory, as well as extensions to topological groups and rings, are accessible. We hope to discuss these and other applications more fully in a later note.

[1] Tychonoff, A., "Uber die topologische Erweiterung von Räumen," *Math. Annalen*, **102**, 544–561 (1930). Alaoglu, L., "Weak Topologies of Normed Linear Spaces," *Ann. Math.*, **41**, 252–267 (1940).

[2] See Alaoglu, *loc. cit.*, p. 256.

[3] Banach, S., "Théorie des Operations Linéaires," 1932, Chapitre VIII.

[4] Banach, *loc. cit.*, pp. 131–132.

[5] The closed convex extension of a compact set in E_T is again compact. See Phillips, R. S., "On Weakly Compact Subsets of a Banach Space," *Amer. J. Math.*, **65**, 108–136 (1943).

[6] Smulian, V., "Über lineare topologische Räume," *Mat. Sbornik N.S.*, **7** (49), 425–448 (1941). See also "Sur les ensembles régulièrement fermes and faiblement-compact dans l'espace du type (B)," *C. R. (Doklady) Acad. Sci. URSS (N.S.)*, **18**, 405–407 (1938).

[7] Banach, *loc. cit.*, p. 80.

[8] Mazur, S., "Über konvex Mengen in linearen normierten Räumen," *Studia Math.*, **4**, 70–84 (1933).

[9] See Kakutani, S., "Weak Topology and Regularity of Banach Spaces," *Proc. Imp. Acad. Tokyo*, **15**, 169–173 (1939).

[10] *Proof:* If X is a limit point of M in ${}_TE^{**}$ and $(f_1, \ldots, f_n)$ are arbitrary elements of E), there exists a sequence (x_m) in M such that $|X(f_i) - f_i(x_m)| < 1/m$ $(i = 1, \ldots, n)$. A standard compactness argument yields an element x in M such that $X(f_i) = f_i(x)$ $(i = 1, \ldots, n)$. But then X lies in E, and hence in M.

[11] See Alaoglu, L., and Birkhoff, G., "General Ergodic Theorems," *Ann. Math.*, **41**, 293–309 (1940).

NOTE ON THE CRITICAL POINTS OF HARMONIC FUNCTIONS

BY J. L. WALSH

Department of Mathematics, Harvard University

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Two theorems recently proved by the writer have wide application in the study of the location of critical points of (*a*) harmonic measure, and (*b*) Green's functions for multiply connected regions of the extended plane, as we wish here to indicate. The present method involves primarily the conformal map onto the interior of a circle of the universal covering surface R^∞ of a given region R, a method used extensively by R. Nevanlinna in the study of harmonic measure.

The first theorem[1] to be applied is

THEOREM 1. *Let C be the unit circle in the w-plane, and let R be the interior of C with the deletion of closed regions bounded by the mutually exterior and disjoint Jordan curves $C_1, C_2, \ldots, C_m$ interior to C. Let $\omega(w, C_1 + \ldots + C_m, R)$ denote the harmonic measure of the set $C_1 + \ldots + C_m$ in the point w of R with respect to R, that is to say, this function ω is harmonic and bounded interior to R, and takes on continuously the boundary values zero on C and unity on $C_1 + \ldots + C_m$. Then the critical points interior to R of $\omega(w, C_1 + \ldots + C_m, R)$ lie in the smallest non-euclidean convex region in C containing $C_1 + \ldots + C_m$.*

The non-euclidean lines interior to C are, of course, the arcs of circles orthogonal to C.

We shall use Theorem 1 in the proof of

THEOREM 2. *Let R be a region bounded by the disjoint Jordan curves $C_1, C_2, D_1, \ldots, D_m$, and let S be the doubly connected region bounded by C_1 and C_2. Then for constant μ, any region $\omega(z, C_1, S) > \mu \geqq 1/2$ or $\omega(z, C_2, S) > \mu \geqq 1/2$ which contains no point of $D = D_1 + D_2 + \ldots + D_m$ contains no critical point of $\omega(z, D, R)$.*

We map conformally the universal covering surface S^∞ of S onto a strip bounded by two parallel straight lines, the latter corresponding to C_1 and C_2, respectively. Each curve D_k is mapped into infinitely many Jordan curves $D_{k1}, D_{k2}, \ldots$, successive curves of which are congruent under a suitable translation independent of k parallel to the sides of the strip. Adjacent to the sides of the original strip are then new strips parallel to them free from points of the images of D.

Map now the parallel strip which is the image of S^∞ onto the interior of the unit circle C in the w-plane. The ends of the strip correspond to two points A and B of C; each line parallel to the sides of the original strip corresponds to a circular arc through A and B Denote by C_1' and C_2' the arcs of C terminated by A and B which are the images of C_1 and C_2, respectively, counted infinitely often. A certain closed lens-shaped region

L interior to C bounded by circular arcs terminating in A and B contains all the images D'_{kq} of the curves D_k; choose L as the smallest such region containing also the circular arc AB orthogonal to C. Then L is non-euclidean convex, and is bounded by arcs $\omega(w, C_1', |w| < 1) = \mu_1 \geqq 1/2$, $\omega(w, C_2', |w| < 1) = \mu_2 \geqq 1/2$. It is not difficult to show that $\omega(z, D, R)$ as interpreted in the w-plane is on any closed set in C exterior to L the uniform limit of the harmonic measure of any finite set $\sum D_{kq}'$ with respect to the interior of C less the closed interiors of that set $\sum D_{kq}'$. Since L contains all the curves $\sum D_{kq}'$, it contains all critical points of this variable harmonic function, and hence L contains all critical points of the limit $\omega(z, D, R)$.

The function $\omega(w, C_j', |w| < 1)$ is automorphic under any map of $|w| < 1$ onto itself which leaves the image of S invariant, and is single-valued on S, so we have $\omega(w, C_j', |w| < 1) = \omega(z, C_j, S)$, for the properties of the harmonic measure define the function uniquely. Theorem 2 now follows at once.

The conclusion of Theorem 2 applies equally well to the location of the critical points of the complementary harmonic measure $\omega(z, C_1 + C_2, R) = 1 - \omega(z, D, R)$.

In the special case that C_1 and C_2 are circles—and this case can always be obtained by a suitable conformal map of S—the loci $\omega(z, C_j, S) = \text{const.}$ are circles of the coaxial family determined by the circles C_1 and C_2; the locus $\omega(z, C_j, S) = 1/2$ is the circle with respect to which C_1 and C_2 are mutually inverse. In this case Theorem 2 is especially simple to apply.

We leave to the reader the details of the proof of a limiting case of Theorem 2:

THEOREM 3. *Let R be a doubly connected region bounded by the Jordan curves C_1 and C_2. Then any region $\omega(z, C_1, R) > \mu \geqq 1/2$ or $\omega(z, C_2, R) > \mu \geqq 1/2$ not containing the point z_0 of R fails to contain the critical point of Green's function $g(z, z_0, R)$ for R with pole in z_0.*

The general method of proof of Theorem 2 applies also if R is bounded by disjoint Jordan curves $C_1, C_2, \ldots, C_n, D_1, D_2, \ldots, D_m$, and we wish to study the location of the critical points of $\omega(z, D, R)$, where $D = D_1 + D_2 + \ldots + D_m$. The case $n = 1$ can be treated by a smooth map of the interior of the circle C of Theorem 1. In the case $n > 2$, as in the case $n = 2$, we map onto the interior of $C: |w| = 1$ the universal covering surface S^∞ of the region S bounded by $C_1, C_2, \ldots, C_n$. In the present case the image of C_k is not one single arc of C, but fills an infinite number of distinct arcs of C which are separated by arcs of C which are images of the other curves C_j. The harmonic measure $\omega(w, C_k', |w| < 1)$, where C_k' is a single arc of C whose points correspond to points of C_k, is not identical with $\omega(z, C_k, R)$; the function $\omega(w, C_k', |w| < 1)$ is automorphic under some but not all substitutions of the group of automorphisms of the function

which maps $|w| < 1$ onto S^∞. The set of images D' of all the curves D_k lies interior to C, and has as limit points on C an infinity of points, the complement of the set of arcs of C (of total length 2π) corresponding to the curves C_k. Denote by R' the image of R interior to C, and by $\bar{R}'$ its closure. Then the function $\omega(z, D, R) = \omega(w, D', R')$ is the limit of the harmonic measure in the point w with respect to the region $|w| < 1$ less the closed interiors of a finite number of the curves D_k' which are the images of the D_k, of that finite number of curves D_k'; this limit is uniform on any closed point set in $\bar{R}'$ containing no limit point of D' on C. Consequently, by Theorem 1 no critical point of $\omega(w, D', R')$ lies in a region $\omega(w, C_k', |w| < 1) > \mu \geqq 1/2$ which contains no point of D', where C_k' indicates not the total image of C_k on C but a single arc of C whose points correspond to C_k traced infinitely many times. Otherwise expressed, all critical points of $\omega(w, D', R')$ lie in any non-euclidean convex region interior to C which contains all points of D'. It is to be noted that non-euclidean geometry defined interior to C enables us to define non-euclidean geometry in S, and that the latter definition is independent of the particular conformal map chosen to map S^∞ onto $|w| < 1$. We state

THEOREM 4. *Let R be a region bounded by the disjoint Jordan curves $C_1, C_2, \ldots, C_n, D_1, D_2, \ldots, D_m$, and let S be the region containing R bounded by $C_1, \ldots, C_n$. Let S^∞ denote the universal covering surface of S, and define non-euclidean lines on S by the conformal map of S^∞ onto the interior of the circle $|w| = 1$. Then any non-euclidean convex region of S which contains all the points of $D = D_1 + \ldots + D_m$ also contains all critical points of $\omega(z, D, R)$. Otherwise expressed, if C_k' is an arc of $|w| = 1$ whose points correspond to points of C_k, then any region $\omega(w, C_k', |w| < 1) > \mu \geqq 1/2$ which contains no image point of D contains no critical points of $\omega(z, D, R)$.*

The conclusion of Theorem 4 applies also to the critical points of $\omega(z, C_1 + \ldots + C_n, R) = 1 - \omega(z, D, R)$.

A limiting case of Theorem 4 can be proved from Theorem 4 itself:

THEOREM 5. *Let R be a region bounded by the disjoint Jordan curves $C_1, C_2, \ldots, C_n$; let R^∞ denote the universal covering surface of R, and define non-euclidean lines on R by mapping R^∞ conformally onto the interior of the circle $|w| = 1$. Then any non-euclidean convex region of R which contains the point z_0 of R also contains all critical points of Green's function $g(z, z_0, R)$ for R with pole in z_0. Otherwise expressed, if C_k' is an arc of $|w| = 1$ whose points correspond to points of C_k, then any region $\omega(w, C_k', |w| < 1) > \mu \geqq 1/2$ which contains no image of z_0 contains no critical points of $g(z, z_0, R)$.*

Theorems 3 and 5 extend at once to a sum

$$g(z, z_0, R) + g(z, z_1, R) + \ldots + g(z, z_n, R)$$

of Green's functions. In this extension of Theorem 3, if all the points z_k lie on $\omega(z, C, R) = 1/2$, so do all critical points of this sum.

The situation of Theorem 4 can be studied by other methods, namely, the conformal map onto a circle of the universal covering surface of the region containing R bounded by any particular subset of the curves C_1, C_2, ..., C_n, D_1, D_2, ..., D_m. We consider in detail the mapping of the universal covering surface R^∞ of R. Here we need the following theorem:

THEOREM 6. *Let α_1, α_2, ..., β_1, β_2, ... be two sets of mutually non-overlapping open arcs of the unit circle $C: |z| = 1$, of total length 2π; we use the notation $\alpha = \alpha_1 + \alpha_2 + \ldots$, $\beta = \beta_1 + \beta_2 + \ldots$. Then no critical points of $\omega(z, \alpha, |z| < 1)$ lie in either of the regions $\omega(z, \alpha_k, |z| < 1) > 1/2$, $\omega(z, \beta_k, |z| < 1) > 1/2$. If α_j and β_k have an end-point in common, no critical points of $\omega(z, \alpha, |z| < 1)$ lie in the region $\omega(z, \alpha_j + \beta_k, |z| < 1) > 1/2$.*

In Theorem 6, two arcs α_j and α_k having an end-point in common can be considered to form a single arc α_e without altering $\omega(z, \alpha, |z| < 1)$ or the conclusion. The conclusion of Theorem 6 obviously applies also to the critical points of $\omega(z, \beta, |z| < 1) = 1 - \omega(z, \alpha, |z| < 1)$.

We omit the proof of Theorem 6; the proof can be given from that of the corresponding theorem[2] concerning a finite number of arcs α_k and β_k. We find at once

THEOREM 7. *Let R be a region bounded by the disjoint Jordan curves C_1, C_2, ..., C_n, D_1, D_2, ..., D_m. Set $C = C_1 + C_2 + \ldots + C_n$, $D = D_1 + D_2 + \ldots + D_m$. Let R^∞ denote the universal covering surface of R, which we map onto the interior of Γ: $|w| = 1$. Then no critical points of $\omega(z, D, R)$ lie in either of the regions $\omega(w, \alpha_k, |w| < 1) > 1/2$, or $\omega(w, \beta_k, |w| < 1) > 1/2$, where α_k is any arc of Γ whose points correspond to points of C, and where β_k is any arc of Γ whose points correspond to points of D.*

This conclusion also applies to the critical points of $\omega(z, C, R) = 1 - \omega(z, D, R)$. The conclusion can of course be expressed in terms of non-euclidean geometry in $|w| < 1$, on R^∞, and in R.

The application of Theorem 7 is of particular interest if Carleman's Principle of Gebietserweiterung is used. Thus let γ be an arbitrary Jordan curve interior to R which intersects no C_k or D_k but separates $C - C_1$ and D from C_1. Denote by S the region bounded by γ and C_1, and by S^∞ its universal covering surface. Then S^∞ lies on R^∞, and in fact an infinity of replicas of S^∞ lie on R^∞. If α_1 is a particular maximal arc of Γ which corresponds to C_1 taken infinitely many times, then a replica of S^∞ corresponds to a region ρ_1 interior to Γ bounded by α_1 and by an arc interior to Γ joining the end-points of α_1. Consequently we have interior to ρ_1

$$\omega(w, \alpha_1, |w| < 1) > \omega(w, \alpha_1, \rho_1),$$

so the region $\omega(w, \alpha_1, \rho_1) > 1/2$ lies interior to the region $\omega(w, \alpha_1, |w| < 1) > 1/2$. In the map of S^∞ onto ρ_1, the function $\omega(w, \alpha_1, \rho_1)$ is single-valued in S, and is equal to $\omega(z, C_1, S)$. Thus we have the

COROLLARY. *Under the conditions of Theorem 7, let γ be an arbitrary Jordan curve interior to R which intersects no C_k or D_k but separates $C - C_1$ and D from C_1, and let S be the subregion of R bounded by C_1 and γ. Then no critical point of $\omega(z, D, R)$ lies in the region $\omega(z, C_1, S) > 1/2$.*

This Corollary is of especial interest when C_1 and γ are circles, for in that case the locus $\omega(z, C_1, S) = 1/2$ is also a circle. In any case, the Corollary yields for every curve C_k (or D_k) an annular region bounded in part by that curve and which is free from critical points of $\omega(z, D, R)$.

Even if C_1 and γ are not circles, suppose a circle γ' in S separates C_1 and γ, and suppose a circle C_1' exterior to S is separated from S by C_1. Let S' denote the annular region bounded by C_1' and γ'. At a point z on γ' or on C_1 we have $\omega(z, C_1', S') < \omega(z, C_1, S)$, so this inequality is valid at every point z common to S and S'. Consequently the locus $\omega(z, C_1', S') > 1/2$, if any, in S is free from critical points of $\omega(z, D, R)$.

Theorem 6 is of obvious significance in the study of the geometric situation of Theorem 7, but where we now study the harmonic measure of an arbitrary set of boundary arcs of R.

THEOREM 8. *Let R be a region bounded by a finite number of disjoint Jordan curves, and let $A_1, A_2, \ldots, A_n$ be a finite number of disjoint Jordan arcs of the boundary of R, with $A = A_1 + A_2 + \ldots + A_n$. Let the images of the arcs $A_1, A_2, \ldots, A_n$ when R^∞ is mapped onto the interior of Γ: $|w| = 1$ be the arcs $\alpha_1, \alpha_2, \ldots$, and let the complementary arcs of Γ be $\beta_1, \beta_2, \ldots$. Any non-euclidean line of the interior of Γ which joins the end-points of an α_k, or the end-points of a β_k, or the extreme end-points of an arc γ of Γ consisting of an arc α_k and an adjacent β_j—such a line separates no critical point of the harmonic measure $\omega(w, \alpha_1 + \alpha_2 + \ldots, |w| < 1)$ from the stated arc which it spans. That is to say, no critical point of this harmonic measure lies in the region $\omega(w, \alpha_k, |w| < 1) > 1/2$, $\omega(w, \beta_k, |w| < 1) > 1/2$, or $\omega(w, \gamma, |w| < 1) > 1/2$. Thus no critical point of $\omega(z, A, R)$ lies in a subregion of R^∞ bounded by a non-euclidean line of R^∞ and either an arc A_k, or an arc B_k of the set of arcs complementary to A, or an arc $A_k + B_j$ consisting of an A_k plus one of the set of complemetary arcs; no such critical point lies in any of the regions $\omega(z, A_k, R^\infty) > 1/2$, $\omega(z, B_k, R^\infty) > 1/2$, $\omega(z, A_k + B_j, R^\infty) > 1/2$.*

Theorem 8 defines a region of R adjacent to every A_k, B_k, and arc $A_k + B_j$, which is free from critical points of $\omega(z, A, R)$. In case one of the Jordan curves, say C_1, bounding R contains but a single one of the arcs A_k, say A_1, we prove that an entire neighborhood of C_1 is thus defined which is free from critical points. Let B_1 denote the arc of C_1 complementary to A_1. An arc of Γ consists of the image of C_1 traced in the same sense infinitely often, hence consists of an infinite number of arcs α_{1k} which are images of A_1 alternating with an infinite number of arcs β_{1k} which are images of B_1. Any such arc α_{1k} can be paired with each of two adjacent arcs β_{1j}, to form an arc γ of Theorem 8; one of the two corresponding

non-euclidean lines on R^∞ commences and terminates at one end-point of A_1, while the other non-euclidean line on R^∞ commences and terminates at the other end-point of A_1; together these two non-euclidean lines bound a neighborhood of C_1 in R free from critical points. But this neighborhood of C_1 is smaller than the neighborhood defined in Theorem 7 and considered in the study of the critical points of $\omega(z, D, R)$. Indeed, on Γ in Theorem 8 each arc γ is a *proper subset* of an arc of Γ which (used in Theorem 7) consists wholly of images of points of C_1; the longer arc determines the larger subregion of $|w| < 1$ free from critical points. Otherwise expressed, each region of Theorem 8 involving γ and free from critical points is defined as $\omega(z, C_1, R^\infty) > 1/2$, where C_1 is counted as a bounding arc of R^∞ but once; the region free from critical points in Theorem 7 is defined as $\omega(z, C_1, R^\infty) > 1/2$, where C_1 is repeated infinitely often as a boundary arc of R^∞; even in Theorem 7 we do not here take *all* points of C_1 in every sheet of R^∞ in defining this harmonic measure.

The Principle of Gebietserweiterung applies also in the case of Theorem 8, where subregions are considered as defined on R^∞. One special case is of fairly wide applicability:

COROLLARY. *Under the conditions of Theorem 8, let the arc E of the boundary of R consist of an arc A_k, or an arc B_k of the set of arcs complementary to A, or an arc A_k plus an adjoining arc B_k; and let a circle (in the extended sense) Ω be divided into arcs Ω_1 and Ω_2 by the end-points of E. Let $E + \Omega_1$ bound a Jordan region in R, and let Ω_2 lie exterior to R. Then no critical point of $\omega(z, A, R)$ lies in the subregion of R if any bounded by E and by the circular arc orthogonal to Ω joining the end-points of E.*

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THE PRODUCTION OF MUTATIONS IN STAPHYLOCOCCUS AUREUS BY IRRADIATION OF THE SUBSTRATE

BY WILSON S. STONE, ORVILLE WYSS* AND FELIX HAAS

THE GENETICS AND THE BACTERIOLOGICAL LABORATORIES, THE UNIVERSITY OF TEXAS

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A knowledge of the gene including its chemistry, function and mutation is of primary importance in understanding life. Muller[1] (see his bibliography also) has summarized the vast collection of information available at present on these subjects. Nevertheless, despite the interest in these problems, little information exists as to the exact chemical nature of any particular gene, much less the differences that exist between genes and

alleles. One method of attack on the nature of the gene system is through a study of mutation. Until Muller[2] showed the effectiveness of x-radiation, no method of producing gene mutations was known. Later it was demonstrated that longer wave length of radiation including ultra-violet would also produce mutations. More recently Auerbach and Robson[3] have demonstrated that mutations may result from the application of certain chemicals (including mustard gas and allied substances).

There are several difficulties in the interpretation of the effect of radiation on the mutation process. First, x-radiation is so drastic a treatment that particular kinds of effects cannot be predicted to the exclusion of others. Second, mutation from irradiation has proved to be a random process. Third, although it has been proved that the mutation rate is directly proportional to the number of individual ionizations, it has not been possible to determine how much of the effect of irradiation resulted from a direct hit on a gene as against an indirect effect from a hit on some other substance in the cell.

Another line of attack has been to compare the effects of x-rays with ultra-violet irradiation, for certain important differences exist in their effects, Stadler.[4] The reasons for these differences have not been completely determined, because here again the material changed directly by the irradiation is unknown. The work on the induction of mutation by chemicals is as yet fragmentary. It should be emphasized that in none of this work has there been evidence for selective induction of particular gene mutations. All agents so far utilized have been *general*, and, therefore, make a study of the particular change involved in mutation exceedingly difficult. As a result there exists no definite knowledge of the chemical or physical change that accompanied any particular mutation.

This paper reports experiments combining irradiation and chemical production of mutations in bacteria. This was accomplished by allowing bacteria to reproduce in a medium containing known substances which had been subjected to irradiation, and then determining if mutations had been induced. The advantage of this method in the chemical analysis of the gene lies in the fact that particular known substances can be irradiated and their effect on gene mutation studied. In addition, the chemical and physical changes that have occurred in those irradiated substances which cause an increase in mutation rate can be investigated.

Experimental.—A 24-hour broth culture of *Staphylococcus aureus* (F.D.A. strain No. 209) was divided into two portions. One half was retained as a control and the other was placed in a petri dish and exposed for 6 minutes at a distance of 50 cm. to radiation produced by a Hanovia double-U SC-2537 ultra-violet mercury vapor lamp operating at 100 milliamperes. Both portions were then plated in appropriate dilutions in nutrient agar and in nutrient agar containing various concentrations of penicillin. After

incubation for 72 hours at 37°C. the colonies were counted. The results are tabulated under Exp. 1 in table 1. All plating was done in triplicate and the figures given are averages of the 3 plates. The control organisms show a distribution of resistant forms similar to that described by Demerec.[5] Although the radiation killed about 40% of the bacteria, a greater number of resistant organisms were present among the survivors than in the unirradiated controls. The exposure to the light produced resistant individuals that had not been present originally.

TABLE 1

INCREASE IN RATE OF MUTATION TO PENICILLIN RESISTANCE BY IRRADIATION OF THE BACTERIA OR THE MEDIUM IN WHICH THEY ARE GROWN

PENICILLIN CONCENTRATION, UNITS/ML.	PLATE COUNT			
	EXP. 1. BACTERIA IRRADIATED		EXP. 2. MEDIUM IRRADIATED	
	CONTROL	IRRADIATED	CONTROL	IRRADIATED
0	221,000,000	135,000,000	114,000,000	46,000,000
0.027	483,000	531,000	27,300	182,300
0.030	86,100	269,800	7,500	40,900
0.033	4,670	56,200	4,610	33,100
0.036	1,250	12,700	2,200	16,900
0.039	1,100	5,180	415	6,500
0.042	495	4,400	317	4,600
0.045	136	1,660	12	1,360

Such individuals are regarded as mutants which differ from the normal population in an alteration of one or several genes. Ultimately, this alteration must be resolved to chemistry and physics. If chemical changes in genes can be brought about by exposure to radiation the possibility is presented that such changes might be introduced into the building materials from which the genes are produced. Therefore, experiments were devised to determine the effect of the irradiation of the substrate upon the mutation rate of organisms subsequently inoculated therein.

Nutrient broth was exposed to radiation from the ultra-violet lamp previously mentioned. After a 3-hour exposure the broth was transferred to a culture flask and an unirradiated equal portion from the same batch of nutrient broth was transferred to a similar flask. Immediately both were inoculated in an identical manner, at the rate of about one million cells per ml. from a 24-hour culture of *S. aureus*. Both flasks were incubated for 5 hours and then appropriate dilutions were plated on nutrient agar containing concentrations of penicillin varying from 0 to 0.045 Oxford units per ml. In all experiments a sufficiently large flask of nutrient agar of each penicillin concentration was prepared so that the plates of both the irradiated and unirradiated series would be poured with agar from the same flask. This eliminates the possibility of errors in dosage at any single level when comparisons are made within any experiment. The plates were counted after incubation for 72 hours at 37°C. and the averages from the triplicate platings are presented as Exp. 2 in table 1.

It is evident that neither culture had attained maximum growth in the 5 hours which had elapsed before plating. The culture growing in the irradiated broth had produced only 46 million cells or about $5^1/_2$ generations, while the culture growing in the control broth produced 114 million cells or about 7 generations. In spite of this lower total production of cells the actual number of resistant bacteria in the irradiated broth at each level of penicillin is many times greater. This fact helps to rule out selection as the cause of the phenomenon and suggests that we are dealing with induced mutation.

This experiment was repeated many times with a number of variations. The penicillin routinely employed was obtained from Commercial Solvents, but Abbott and Merck penicillins were also used. Another strain of *S. aureus* was substituted for the F.D.A. strain in a few of the experiments. For many of the tests a Hanovia analytical model quartz-mercury lamp with Type A burner was used at a distance of 20 cm. and the irradiation of the broth was continued for only 90 minutes. In most cases water lost from the broth by evaporation during exposure to the lamp was replaced, but in some cases it was not. The size of inoculum was varied and the time of plating was delayed to as long as 30 hours after inoculation. Although some quantitative differences were noted the results obtained in all cases were qualitatively similar, *viz.*, cultures grown in irradiated broth produced more mutations.

TABLE 2

INCREASE IN MUTATION RATE BY IRRADIATION OF COMPONENTS OF A SYNTHETIC MEDIUM

PENICILLIN CONCENTRATION, UNITS/ML.	PLATE COUNTS ON CULTURES GROWN IN A SYNTHETIC MEDIUM AFTER IRRADIATION OF THE INDICATED COMPONENTS			
	NONE (CONTROL)	COMPLETE MEDIUM	MINERAL SALTS	AMINO ACIDS AND VITAMINS
0	800,000,000	No growth	900,000,000	195,000,000
0.04	33,000	No growth	40,000	150,000
0.07	4	No growth	4	196
0.10	1	No growth	1	16

If the synthetic medium described by Fildes, *et al.*,[6] is subjected to the same irradiation as the nutrient broth in Exp. 2, table 1, the organisms make no growth. This medium consists of mineral salts, the amino acids found in casein, glucose, and the vitamins thiamin, niacin and biotin. In our experiments we supplemented the latter with one mg. per liter each of adenine, guanine and uracil. Each component was prepared in sterile solution at several times the final concentration so that it could be irradiated separately before combining with the other constituents of the medium. In the experiment reported in table 2, four batches of the synthetic medium were prepared: (1) an unirradiated control, (2) the complete medium irradiated, (3) a double strength solution of the mineral salts irradiated and then added to the remaining components, (4) a triple strength

solution of the amino acids and vitamins irradiated and added to the remaining components. The four media were inoculated in an identical manner and incubated for 24 hours. At that time the culture in which all the components were irradiated had made no visible growth so plate counts were made only on the other three cultures. Irradiation of the mineral salts resulted in a mutation rate no higher than the control but irradiation of the amino acids and vitamins resulted in a marked increase in the mutation rate. Further experiments showed that a good part of the toxic effect (which prevented growth when the complete synthetic medium was subjected to the amount of radiation routinely used in nutrient broth experiments) arose from the glucose. When the irradiation of the glucose was decreased to a level that permitted growth, the mutation rate of cultures grown in media prepared from it was not enhanced.

That the irradiation of the amino acids alone increased the mutation rate is indicated in table 3. Nutrient broth cultures were included in this experiment because the plating was done on agar containing streptomycin as well as on agar containing penicillin. It is evident that rate of mutation

TABLE 3

INCREASE IN THE RATE OF MUTATION TO STREPTOMYCIN AND PENICILLIN RESISTANCE

INHIBITOR, UNITS/ML.	NUTRIENT BROTH		SYNTHETIC MEDIUM	
	CONTROL	IRRADIATED	CONTROL	AMINO ACIDS IRRADIATED
0	300,000,000	260,000,000	1,250,000,000	900,000,000
Penicillin				
0.04	13,000	120,000	12,000	55,000
0.07	10	310	30	1,520
Streptomycin				
1.0	42,000	140,000	30,000	168,000
3.0	5,000	33,000	2,700	23,000

to streptomycin resistance follows the same pattern observed with penicillin resistance. The streptomycin employed in the experiment was crystalline material obtained from Merck which had a potency of 187 units per mg. Other experiments were carried out with lyophilized powder from Commercial Solvents put up in ampules containing 100,000 units.

To demonstrate that the streptomycin- and penicillin-resistant organisms were the result of different mutations, a number of colonies of the mutants were picked and transferred to nutrient agar. Similarly, a number of subcultures were isolated from the control plates. After 24-hour incubation a uniform inoculum from each isolate was streaked to sectors of plates containing streptomycin or penicillin. The plates were incubated for 48 hours and the growth of each culture on each concentration of antibiotic was noted (table 4). Due to the small inoculum used the results were generally clear-cut, although occasionally only a few colonies appeared;

these were reported as positive growth. It will be observed that mutants selected for penicillin resistance differ greatly from the controls when tested on penicillin agar but are very similar to the control organisms when tested on streptomycin agar. The streptomycin-resistant mutants differ from both the controls and the penicillin-resistant mutants. The mutant population is composed of different individuals whose gene differences are induced during growth in the irradiated substrate; the specific mutants are then separated from each other and from the normal population by plating on agar containing the inhibiting substances.

After the specificity of the resulting mutants was demonstrated, attempts were made to determine if specificity could be introduced into the induction of mutation, i.e., if the rate of mutation of one particular gene could be influenced without affecting the rate of mutation of others. Preliminary experiments indicate that this may be possible, for by suitable irradiation procedures we have been able to increase the rate of mutation to penicillin resistance without affecting the rate of mutation to streptomycin resistance.

TABLE 4

SPECIFICITY OF THE DRUG-RESISTANT MUTANTS

STRAINS	NO. TESTED	NO. OF STRAINS GROWING ON MEDIUM CONTAINING: PENICILLIN (UNITS/ML.) 0.05	0.1	0.15	1.0	STREPTOMYCIN (UNITS/ML.) 3	5	10
Penicillin-resistant	21	21	15	9	1	14	6	1
Controls	24	13	1	0	0	12	7	2
Streptomycin-resistant	29	12	2	0	0	29	29	24

Discussion.—These data are the preliminary results of the new method of investigation of gene mutation and gene chemistry. Irradiation is used to *activate* selected chemicals, which, when utilized by the cells, cause mutations. These substances might be termed *activated mutators*. The experimental methods used are very effective in detecting mutations to resistance to toxic agents, in this case penicillin and streptomycin.

Several lines of evidence indicate that these are induced mutations, not the result of selection:

1. In an immature culture there are numerically many more mutants in the irradiated broth despite a smaller total population (table 1).

2. When mutant strains were isolated and their rate of growth was followed in both irradiated and unirradiated broth it was found that they grew more slowly in the former; the rate of growth in both media did not differ significantly from that of control organisms.

3. These mutations are of independent origin and do not represent a general increase in resistance to toxic agents for, on testing, the penicillin-

resistant mutants are no more resistant to streptomycin than are the controls and vice versa (table 4).

It seems improbable that this method of treatment is limited to the production of mutations concerned with resistance. The first two things tried—mutations to resistance to penicillin and streptomycin—were induced by this treatment. With suitable tests many other types of mutation should be detected. Therefore, we believe this will prove to be a generally useful procedure, within its limitations, when used to produce other types of mutation, and mutations in other organisms.

Little need be said of the obvious advantage of this method for the study of mutation and gene chemistry. Instead of treating an exceedingly heterogeneous living organism, we are treating a selected chemical and determining if it produces mutations. If it does, we can investigate the physical or chemical changes that have occurred. These may be either or both of the following: (1) The production of different chemical compounds under the influence of irradiation. (2) Some mechanism involving a shift to a higher energy level by the absorption of a quantum of energy and subsequent effects of this energy transfer. At present we cannot decide between these alternatives, although we have determined that the mutating ability of the treated material is reduced on aging and more rapidly by heat treatment. This suggests that alternative (2) may be correct, but of course it only concerns these particular mutations. On the other hand, it is known that a definite chemical substance, nucleic acid, can induce transformation in the genetic material of microörganisms (Avery, *et al.*[7]) The possibility of producing selected mutations offers a fascinating field for further investigation. Certainly the possibility is inherent in this method. Genes must differ; therefore, we may be able to select agents which will affect only one or at most a few genes and so, indirectly, study gene chemistry. In the experiments reported here, we were able to produce two types of resistant mutants. The difference in survival in the different concentrations of the antibiotics indicates that several different alleles or different mutations are involved (tables 1, 2, 3). We have not been able to determine if the treatment produces changes in an existing gene or if it causes an error in its autocatalytic reproduction. Under ordinary circumstances genes are remarkably free from errors in copy as exemplified by the low rate of spontaneous mutation.

There are several general considerations which follow from these discoveries. For example, a study of the effect of irradiated substrate on the development of cancerous tissue may prove profitable. If any of the activated mutators prove stable this phenomenon must be considered in nutrition and in safeguarding the use of atomic energy. The most important consideration is the possibilities opened for further investigation into the gene and mutation. These experiments have shown that at least part of

the mutations produced by irradiation may be the result of an indirect effect in addition to those from a direct hit on the gene. They may explain at least part of the discrepancy between the observed normal mutation rate and that which has been calculated as attributable to direct hits on genes, Muller.[1] Natural radiation is much more important in the mutation process if it can induce mutation by an effect on the food as well as on the organism.

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